Chlorinated Hydrocarbons in Women with Repeated Miscarriages

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This study was conducted to investigate a possible etiological role of chlorinated hydrocarbons in the pathogenesis of repeated miscarriages. The blood levels of chlorinated hydrocarbons [CHCs: pentachlorophenol, hexachlorocyclohexane, hexachlorobenzene, the dichlorodiphenyltrichloroethane (DDT) group, polychlorinated biphenyls] were determined in 89 women with repeated miscarriages, who were referred to the University Hospital of Obstetrics and Gynecology of Heidelberg for investigations between 1989 and 1993, and compared to a previously investigated reference population. In more than 20% of the women, at least one of the CHC levels exceeded the reference range. CHC levels did not differ significantly between women with primary or secondary and early or late miscarriages; neither did they differ between women with hormonal or immunological disorders as causes of repeated miscarriages or women with idiopathic repeated miscarriages. No significant associations were detected between CHC levels and further conceptions or the outcome of further pregnancies. As significant associations were found between increasing CHC blood concentrations and immunological and hormonal changes, CHCs may have an impact on the pregnancy course in certain cases. *Key words:* chlorinated hydrocarbons, infertility, repeated miscarriages. *Environ Health Perspect* 106:675–681 (1998). [Online 16 September 1998]
http://ehpnet1.niehs.nih.gov/docs/1998/106p675-681gerhard/abstract.html

Chlorinated hydrocarbons (CHCs) are ubiquitous persistent substances that are incorporated into the body via ingestion, inhalation, and transcutaneously. Cell and animal studies have shown that CHCs are stored in different organs, especially the fatty tissue; exhibit hormonelike activity; and induce immunological changes (*1–5*). Miscarriages, fetal malformations, and growth retardation were observed in women with occupational exposure to chlorinated hydrocarbons or accidental CHC poisoning (*6–10*).

In women with repeated miscarriages, intensive diagnostics allow the identification of a definite cause in some cases only, such as, for example, chromosomal, uterine, or hormonal disorders. In the majority of miscarriages, however, the cause remains unknown, so that discrete hormonal or immunological disorders are assumed. Therefore, further factors must be identified that may affect female fertility and play an etiological role in the pathogenesis of repeated miscarriages. In a previous cross-sectional study of an unselected group of 322 infertile women, increasing blood dichlorodiphenyldichloroethene (DDE) levels were significantly associated with a decreasing conception rate and increasing pentachlorophenol (PCP) concentrations with an increasing miscarriage rate (*11*).

We conducted this observational study to detect a possible etiological role of chlorinated hydrocarbons in the pathogenesis of repeated miscarriages. CHC levels of women with repeated miscarriages were compared with those of a reference population. Furthermore, women with primary versus secondary miscarriages, women with early versus late miscarriages, and women with hormonal or immunological disorders as causes of repeated miscarriages versus women with idiopathic repeated miscarriages were investigated in order to detect possible differences between these different types of repeated miscarriages. The working hypothesis was that an increased body burden of CHC may be an important etiological factor in repeated miscarriages. We therefore expected to detect increased CHC levels in women with repeated miscarriages in comparison to the reference population. We also investigated possible effects of low-level CHC exposure on hormonal and immunological parameters, which may also play an etiological role in repeated miscarriages. The long-term effects of CHC levels with respect to further conceptions and the outcome of pregnancies were also observed. We must stress, however, that this is an observational study conducted to detect possible associations and to create new working hypotheses for controlled studies.

Material and Methods

Subjects. We determined CHC levels in addition to the usual diognostics in 89 women with a history of at least two miscarriages who attended the Clinic of Reproductive Endocrinology between 1989 and 1993. Miscarriages in women who had never delivered a baby are defined as primary miscarriages (n = 65). A history of miscarriages following the delivery of at least one baby with the same partner defines secondary miscarriages (n = 24). Sixty women complained of early miscarriages (by the 12th week of gestation), and 5 women complained of late miscarriages (13th–25th week of gestation). Twenty-four women had a history of both types. Thirty-nine percent of the women had a history of 2 miscarriages, 31% of 3, and 30% of 4 or more miscarriages, with a maximum 12 miscarriages. The median age of the women was 28 years (range 21–39 years), the median body mass index (BMI = body weight/height^2) was 22 kg/m^2 (range 17–37 kg/m^2). Of these women, 87% were German. Non-Germans were mainly women from Eastern and Central Europe. All of them were Caucasian. Seventy-two percent of the women were employed, 15% were housewives, and 13% were industrial workers. Seventy percent of the women were nonsmokers, 18% smoked 1–10 cigarettes/day, 8% smoked 11–20 cigarettes/day, and 4% smoked more than 20 cigarettes/day. All subjects were medically fit at the time of this study. The menstrual cycles were found to be eumenorrhoeic (interval 25–30 days) in 70% and oligomenorrhoeic (interval 31 days–6 weeks) in 20%. Severe menstrual cycle disturbances (interval >6 weeks) were found in 10%. These women had wished for a child for less than 3 years (40%), 3–5 years (40%), or more than 5 years (20%).

Investigations. Chromosomal, uterine, medical, or immunological causes of miscarriages were investigated with help of the usual diagnostic procedures (*12*), e.g., chromosomal analysis, hysterosalpingography, determination of autoantibodies, etc. The hormonal tests were performed in the early follicular and the luteal phases of the menstrual cycle [determinations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) at baseline and following the administration of gonadotrophin-releasing hormone (GnRH); prolactin and thyroid-stimulating hormone (TSH) at baseline and at 30 min after stimulation with thyrotropin-releasing homone (TRH); testosterone; dehydroepidandrosterone sulfate (DHEAS); 17β-estradiol; progesterone].

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Received 29 January 1998; accepted 28 May 1998.

Table 1. Chlorinated hydrocarbon (CHC) blood levels in 89 patients with repeated miscarriages

Chlorinated hydrocarbon	Reference level	Mean ± SD	Minimum	25th percentile	Median	75th percentile	Maximum	Percent above reference
Pentachlorophenol (µg/l)	<25	15 ± 1	3	8	11	18	95	15
Total polychlorinated biphenyls (ng/l)	<1,500	792 ± 68	121	419	718	913	4,372	22
Dichlorodiphenyltrichlorethane (ng/l)	<2,500	1,244 ± 153	10	385	826.5	1,629	8,593	15
β-Hexachlorocyclohexane (ng/l)	<700	273 ± 19	10	172	214	300	950	7
γ- Hexachlorocyclohexane (ng/l)	<100	47 ± 3	15	26	44	63	132	10
Hexachlorobenzene (ng/l)	<1,000	679 ± 90	106	290	500	835	7,046	15
CHC score		14 ± 2	6	11	15	17	24	

SD, standard deviation.

Table 2. Correlations between serum pentachlorophenol concentrations and hormonal or immunological parameters

Parameter	No.	r^a	Group[b]	*p*-Value
Hormones				
LH (30 min)[c]	33	0.40382	Early miscarriages ($n = 60$)	0.0198
Testosterone	58	-0.26356	Primary miscarriages ($n = 65$)	0.0465
Triiodothyronine	24	-0.50970	Late miscarriages ($n = 29$)	0.0110
Immunology				
IgG	18	-0.54855	Secondary miscarriages ($n = 24$)	0.0184
Lymphocytes	55	-0.28032	Primary miscarriages	0.0382

[a]Spearman correlation coefficient *r*.
[b]Total group of women with repeated miscarriages ($n = 89$).
[c]Luteinizing hormone 30 min after stimulation with gonadotrophin-releasing hormone.

Table 3. Correlations between blood β-hexachlorocyclohexane concentrations and hormonal or immunological parameters

Parameter	No.	r^a	Group	*p*-Value
Hormones				
Testosterone	56	-0.34427	Early miscarriages ($n = 60$)	0.0094
TSH (30 min)[b]	57	-0.27908	Early miscarriages	0.0355
Immunology				
Neopterine	22	-0.43788	Total group ($n = 89$)	0.041

[a]Spearman correlation coefficient *r*.
[b]Thyroid-stimulating hormone 30 min after stimulation with thyrotropin-releasing hormone.

The immune systems of the women were evaluated by determining the lymphocyte subpopulations, neopterine, and the immunoglobulins IgG, IgM, and IgA (*13*).

The following causes of repeated miscarriages were identified in the women: 1) hormonal disorders were found in 31% [hyperprolactinemia in 9% (prolactin >450 IU/l), hyperandrogenemia in 7% (testosterone >500 pg/ml and/or DHEAS >4,500 ng/ml), combined hyperprolactinemia and hyperandrogenemia in 1%, and luteal insufficiency in 14% (repeated luteal progesterone <10 ng/ml]; 2) 5 women had uterine abnormalities (uterus subseptus in 2, uterus bicornis in 2, and uterine fibroids in 1); 3) 1 woman had chromosomal translocation; 4) the antiphospholipid syndrome was found in 10 women [partial thromboblastin time (PTT) >30 sec and/or anticardiolipin antibodies >6 U/ml); and 5) 8 subjects had positive autoantibodies with uncertain pathogenetic relevance [immunologically not classified; human leukocyte antigen (HLA) antibodies in 1 woman, cross-match positive in 3, antinuclear antibodies (ANA) in 2, and anti-DNA antibodies in 2]. In 43% of the women, none of the above causes was detected (idiopathic miscarriages). The conception and miscarriage rates were observed in the women over a period of 1–5 years following the above diagnosis. In women with a known cause of repeated miscarriages, individual therapy was given as previously described (*11,14*): hormonal therapy was used to improve follicular maturation and luteal function; progesterone was administered during the first trimester of pregnancy; vitamins, minerals, and trace elements were given if needed; uterine abnormality was surgically corrected if nesessary; and acetylsalicylic acid was administered to subjects with antiphospholipid syndrome. In one-third of the subjects, no therapy was given because the women refused any treatment or a specific cause of repeated miscarriages was not identified.

CHC determination. Blood (30 ml) was collected into a tube containing EDTA after an overnight fast. The glass vessels for blood collection were specially cleaned and sterilized (*15*). The samples were sent to the laboratory (K. Bauer, Saarbrücken, Germany) by courier and processed immediately. Analyses were performed as previously described using gas chromatography and, for different values, with mass spectrometry (*16*). The following CHCs were determined (reference values): α-hexachlorocyclohexane (α-HCH) (<10 ng/l), β-HCH (<700 ng/l), γ-HCH (lindane; <100 ng/l); hexachlorobenzene (HCB; <1,000 ng/l); PCP (<25 µg/l); and polychlorinated biphenyls (PCBs) International Union of Pure and Applied Chemistry (IUPAC) numbers 28, 52 (<10 ng/l), 101 (<100 ng/l), 138 (<500 ng/l), 153 (<600 ng/l), and 180 (<300 ng/l). The sum of the congeners 101–180 was used for evaluation because only these substances were found in significant concentrations (<1,500 ng/l). Because α-HCH and PCBs 28 and 52 were always below the level of detection (10 ng/l), they were not considered for evaluation. Only DDE, as the storage form of DDT, was detectable in significant concentrations and was used for evaluation (<2,500 ng/l). The CHC score was calculated as follows: each subject scored 1–4 points for the blood levels of PCP, PCB, DDE, β-HCH, γ-HCH, and HCB (1 point: level <25th percentile, 2 points: level 25th–50th percentile, 3 points: 50th–75th percentile, 4 points: 75th–100th percentile). Therefore, the minimum score was 6 and the maximum score was 24.

Statistical analyses. Statistical analyses were performed first on the absolute CHC concentrations. Correlation analysis according to Spearman was performed for continuous variables. Because the values were not normally distributed, the Wilcoxon test was applied to compare continuous variables with discrete variables and to compare two unpaired variables. For three unpaired variables, the Kruskal-Wallis test was used. Values in the text refer to the median and quartile difference. The chi-square test was used for the comparison of two discrete variables. Because this was an exploratory study to form different hypotheses, we did not adjust α despite the large amount of different calculations. Partial analysis was used to exclude the influence of age and BMI. The level of significance was $p<0.05$.

Only the significant associations independent of age and BMI are shown in the tables.

Results

We found no significant differences between women with early or late and primary or secondary repeated miscarriages with respect to blood CHC levels or clinical and laboratory parameters. We found the CHC concentrations to be within the reference range in 80% of the women (Table 1). Among the different CHCs, PCB levels were increased most frequently and β-HCH concentrations most rarely. Twenty-six women had elevated levels of one of the different CHCs, 12 subjects had two, 1 subject had three, and 4 women had four. In smokers, increased CHC levels were not detected. By contrast, nonsmokers had significantly higher β-HCH concentrations than smokers; median and quartile differences were 229 and 249 ng/l versus 200 and 90 ng/l for nonsmokers and smokers, respectively (p = 0.0225). We found no associations between CHC concentrations and the parameters age, body height or weight, BMI, occupation, age at menarche, and duration of the unfulfilled wish for a child. Foreigners had significantly higher concentrations of DDE than German women (1,697 and 2,121 ng/l vs. 681 and 11 ng/l, respectively; p =0.0035). Women with menstrual cycle abnormalities had significantly greater levels of PCP than eumenorrhoeic subjects (15 and 7 µg/l vs. 10 and 8 µg/l; p = 0.0233). Significantly greater PCP levels were also found in women with a history of at least three miscarriages compared to subjects with two miscarriages (21 and 16 µg/l vs. 10 and 8 µg/l; p = 0.03). Women with a history of at least four miscarriages (n = 25) had significantly greater concentrations of total PCBs (797 and 535 ng/l vs. 536 and 557 ng/l; p = 0.0386), γ-HCH (52 and 36 ng/l vs. 28 and 22 ng/l; p = 0.0075), and HCB (772 and 654 ng/l vs. 390 and 445 ng/l; p = 0.0375) than the other women.

The significant associations between PCP levels and hormonal or immunological parameters are summarized in Table 2. In women with early miscarriages, the stimulated LH peak was significantly greater with increasing PCP levels. In subjects with primary miscarriages, testosterone levels were found to be lower when PCP concentrations were higher. In women with late miscarriages, an inverse correlation was found between triiodothyronine (T_3) levels and PCP. With increasing PCP concentrations, decreasing lymphocyte counts were noted in subjects with primary miscarriages and decreasing IgG levels were seen in subjects with secondary miscarriages.

Table 4. Correlations between blood γ-hexachlorocyclohexane concentrations and hormonal or immunological parameters

Parameter	No.	r^a	Group	p-Value
Hormones				
Testosterone	83	-0.30342	Total group (n = 89)	0.0053
	22	-0.54042	Secondary miscarriages (n = 24)	0.0094
	56	-0.34809	Early miscarriages (n = 60)	0.0086
TSH	84	-0.32937	Total group	0.0022
	62	-0.30496	Primary miscarriages (n = 65)	0.0159
	57	-0.34543	Early miscarriages	0.0085
TSH (30 min)[b]	57	-0.36870	Early miscarriages	0.0048
FSH	63	-0.25055	Primary miscarriages	0.0476
Estradiol (follicular)	19	0.43197	Secondary miscarriages	0.0048
Immunology				
Total lymphocytes[c]	19	0.63153	Secondary miscarriages	0.0037
CD 3[d]	19	0.65261	Secondary miscarriages	0.0025
CD 4[e]	19	0.56917	Secondary miscarriages	0.0110
CD 8[f]	55	0.28250	Early miscarriages	0.0366
CD 11b[g]	54	0.35266	Early miscarriages	0.0089

Abbreviations: TSH, thyroid-stimulating hormone; CD, clusters of differentiation; FSH, follicle-stimulating hormone.
[a]Spearman correlation coefficient r.
[b]TSH 30 min after stimulation with thyrotropin-releasing hormone.
[c]Total lymphocytes per microliter of blood.
[d]CD 3 = Total T cells per microliter of blood.
[e]CD 4 = T-helper cells per microliter of blood.
[f]CD 8 = T-suppressor cells per microliter of blood.
[g]CD 11b = monocytes and natural killer cells per microliter of blood.

Table 5. Correlations between blood hexachlorobenzene concentrations and hormonal or immunological parameters

Parameter	No.	r^a	Group	p-Value
Hormones				
FSH	27	0.38076	Late miscarriages (n = 29)	0.050
Immunology				
CD 8[b]	25	-0.43663	Late miscarriages	0.0291
CD4/CD8-ratio[c]	24	0.50098	Late miscarriages	0.0126
Neopterine	21	-0.46568	Total group (n = 89)	0.0334
	16	-0.50301	Early miscarriages (n = 60)	0.0470

Abbreviations: FSH, follicle-stimulating hormone; CD, clusters of differentiation.
[a]Spearman correlation coefficient r.
[b]CD 8 = T-suppressor cells per microliter of blood.
[c]T-helper cell/T-suppressor cell ratio.

In women with early miscarriages, decreasing testosterone and decreasing stimulated TSH concentrations were noted the higher the β-HCH levels (Table 3).

As for PCP and β-HCH, inverse correlations were found between testosterone and γ-HCH levels (Table 4). γ-HCH concentrations were also inversely correlated to FSH and TSH levels. Early follicular estradiol concentrations were higher with increasing γ-HCH levels. In women with early and secondary miscarriages, different lymphocyte subsets were directly correlated to γ-HCH levels (Table 4).

In women with late miscarriages, increasing FSH concentrations and an increasing T-helper cell/T-suppressor cell ratio due to decreasing T-suppressor cell counts were noted as HCB levels increased (Table 5).

Marked associations to different hormonal parameters were detected for PCBs (Table 6): higher FSH levels were found with increasing PCB levels (as seen with HCB) and decreasing TSH levels (as seen with γ-HCH). LH and prolactin were directly correlated to PCB levels. Various associations between PCBs and immunological parameters were noted, especially in women with late miscarriages: an inverse correlation to the leukocyte count and direct correlations to IgM and clusters of differentiation (CD) 11b- and CD 16-positive cells.

DDE was inversely correlated to TSH levels and directly correlated to follicular estradiol concentrations (Table 7). A direct correlation was found between DDE and the lymphocyte count. In women with early miscarriages, DDE levels were directly correlated to the counts of total T cells and the T helper cells. In the total group of women with repeated miscarriages, the count of interleukin 2 receptor-positive cells decreased and the monocyte and natural killer cell count increased as DDE concentrations increased.

With increasing CHC scores, FSH and prolactin increased and testosterone levels decreased (Table 8).

A total of 75 women conceived during the observation period. The overall miscarriage rate was 30%. No significant differences in CHC levels were found in the different therapy groups, i.e., women with hormonal or immunological disorders as causes of repeated miscarriages, women with idiopathic miscarriages, women who conceived or not, women who miscarried again, or the subjects who delivered a baby.

Table 6. Correlations between blood polychlorinated biphenyl concentrations and hormonal or immunological parameters

Parameter	No.	r^a	Group	*p*-Value
Hormones				
FSH	22	0.46936	Secondary miscarriages (*n* = 24)	0.0275
LH	21	0.44018	Secondary miscarriages	0.0458
Prolactin	84	0.23165	Total group (*n* = 89)	0.0340
	23	0.82016	Secondary miscarriages	0.0001
	57	0.30174	Early miscarriages (*n* = 60)	0.0225
TSH	82	-0.30776	Total group	0.0049
	61	-0.32469	Primary miscarriages (*n* = 65)	0.0107
	55	-0.31410	Early miscarriages	0.0195
TSH (30 min)[b]	55	-0.30144	Early miscarriages	0.0253
Immunology				
Leukocytes	56	-0.28501	Early miscarriages	0.0332
	26	-0.47759	Late miscarriages (*n* = 29)	0.0136
Monocytes	51	-0.31991	Early miscarriages	0.0221
CD 11b[c]	78	0.26446	Total group	0.0193
	25	0.39846	Late miscarriages	0.0485
	59	0.29563	Primary miscarriages	0.023
CD 11b %[d]	59	0.25975	Primary miscarriages	0.0469
CD 16[e]	25	0.47788	Late miscarriages	0.0157
CD 25[f]	29	-0.42069	Total group	0.0231
Neopterine	17	-0.48732	Early miscarriages	0.0472
IgM	22	0.46766	Late miscarriages	0.0282

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone; CD, clusters of differentiation.

[a]Spearman correlation coefficient *r*.

[b]TSH 30 min after stimulation with thyrotropin-releasing hormone.

[c]CD 11b = monocytes and natural killer cells per microliter of blood.

[d]Percentage of CD 11b-positive cells.

[e]CD 16 = natural killer cells per microliter of blood.

[f]CD 25 = interleukin 2 receptor-positive cells per microliter of blood.

Table 7. Correlations between blood dichlorodiphenyltrichlorethane concentrations and hormonal and immunological parameters

Parameter	No.	r^a	Group	*p*-Value
Hormones				
TSH	84	-0.32937	Total group (*n* = 89)	0.0022
Estradiol (follicular)	19	0.55145	Secondary miscarriages (*n* = 24)	0.0144
Immunology				
Lymphocytes	74	0.23761	Total group	0.0415
	52	0.30857	Primary miscarriages (*n* = 65)	0.0260
CD 3[b]	50	0.28682	Early miscarriages (*n* = 60)	0.0434
CD 4[c]	51	0.28691	Early miscarriages	0.0412
CD 11b[d]	75	0.27335	Total group	0.0176
CD 25[e]	28	-0.43788	Total group	0.0198

Abbreviations: TSH, thyroid-stimulating hormone; CD, clusters of differentiation.

[a]Spearman correlation coefficient *r*.

[b]CD 3 = total T cells per microliter of blood.

[c]CD 4 = T-helper cells per microliter of blood.

[d]CD 11b = monocytes and natural killer cells per microliter of blood.

[e]CD 25 = interleukin 2 receptor-positive cells per microliter of blood.

Table 8. Correlations between chlorinated hydrocarbon score and hormonal parameters

Hormones	No.	r^a	Group	*p*-Value
FSH	78	0.22632	Total group (*n* = 89)	0.0463
Prolactin	79	0.22998	Total group	0.0415
Testosterone	75	-0.23126	Total group	0.0459

FSH, follicle-stimulating hormone.

[a]Spearman correlation coefficient *r*.

Discussion

In this specific group of women with repeated miscarriages, the contamination with certain CHCs was more pronounced than in the total population, which was investigated in a group of more than 1,900 women of the same region. In 20% of the women with repeated miscarriages, at least one of the CHCs was increased above the reference range (*15*). A similar frequency of increased CHC levels was previously noted in a group of women with infertility (*11*). The CHC levels, especially PCP, HCB, and PCB, were found to be increased above the reference range in 6–15% of the women. Although DDT has been banned in most countries for some years now, relatively high blood DDE concentrations were observed. In 14% of the women, levels above the reference range were found. These women were mainly non-Germans who may have had a greater exposure in their youth. Furthermore, they may still be exposed to DDT due to the use of imported food from their home countries. Only for DDE were significantly higher levels found in foreign compared to German women. There was a tendency toward higher PCP and β- and γ-HCH levels in foreign women and a tendency toward higher PCB levels in German women, but these levels did not reach statistical significance. This pattern is nevertheless typical for our geographical region (*17*).

No significant age dependency was noted for the different CHCs, which may be due to the narrow age range of 21–39 years in the women investigated. In populations with a greater age range, the contamination with CHCs with long half-lives, such as HCH, PCB, and DDE, has been found to be age dependent (*11,18*). Higher blood β-HCH levels were noted in nonsmokers than in smokers. In a study of mother's milk samples, inverse associations between smoking and HCH, PCB, and DDE levels have been previously noted (*19*). Smoking may lead to an induction of detoxicating enzyme systems resulting in a more pronounced β-HCH degradation.

Higher PCP concentrations were noted in women with menstrual cycle abnormalities than in eumenorrhoeic women. This may be due to hormonal changes, which are discussed below. After adjustment for age, it was noted that women with a history of at least three miscarriages had markedly greater PCP levels than women with two miscarriages. In an unselected population of more than 300 women, an increasing miscarriage rate has been previously noted with increasing PCP levels (*17*). Especially women with exposure to wood preservatives in their home and work place were affected (*20*).

Embryotoxic effects of PCP have been confirmed in numerous animal feed studies and may also contribute to repeated miscarriages in humans (*21–25*). A source of PCP exposure in their surroundings was always identified in women with repeated miscarriages who had PCP levels greater than 25 µg/l. PCP levels did not differ between women who miscarried again and subjects who delivered a baby in this study. This may be because women with increased PCP levels were encouraged to eliminate PCP exposure by renovating their homes. Once the PCP levels returned to normal, further conceptions were encouraged in the women.

Different CHC levels (PCB, γ-HCH, HCB) were higher in women with a history of more than three miscarriages than in women with two miscarriages. The CHC body burden of women with three miscarriages may have reached an embryotoxic level, as previously observed in animal feed studies (*1,5,21,26*).

CHC levels did not differ in women with early or late and primary or secondary miscarriages; they also did not differ in women with hormonal or immunological disorders as causes of repeated miscarriages or in women with idiopathic miscarriages. In animal studies, feeding of different CHCs resulted in different pregnancy complications or conditions such as infertility, early or late miscarriages, fetal growth retardation, or death. Higher levels of organochloric compounds and PCBs have been found in women with miscarriages compared to women with a normal course of pregnancy (*27,28*). In women with occupational PCB exposure who successfully carried their babies to term, infants had lower birth weights than controls (*29*). Whether a toxic substance affects the course of pregnancy in the individual case depends on numerous additional factors such as genetic disposition, diet, alcohol use, smoking habits, and contamination with other harmful substances, which are difficult to account for in animal studies.

For all CHCs, significant associations to the endocrine system were found, although little is known about the exact mechanism of these assumed effects. The main correlations concerned the thyroid and pituitary axis, as well as testosterone levels. The associations found in our study may indicate real effects of CHCs on these hormonal systems. PCP has been found to inhibit the enzyme sulfatase, so that less thyroxine is converted into T_3, which may account for the inverse correlation between PCP and T_3 levels in our population (*30*). PCP-containing wood preservatives also contain dioxins and furans, which have been found to affect the thyroid function (*31–34*). We found inverse associations between TSH and the CHCs β-HCH, γ-HCH, PCB, and DDE. For PCBs, thyroxinelike effects have been shown to result in the suppression of TSH (*30*). HCH may also induce hypothyroidism (*35*). Chlorophenols affect the availability and levels of biologically active free thyroxine through interaction with thyroxine-binding proteins (*36*). PCP, β-HCH, γ-HCH, and the CHC score were age-independently inversely correlated to testosterone levels. The adrenal steroid DHEAS was not affected and testosterone production essentially takes place in the ovary; thus, this may indicate an ovarian CHC toxicity. For PCBs, the decreased testosterone levels were accompanied by increased levels of FSH and LH, which may be secondary and confirmatory of the direct ovarian toxicity (*37*). In human cumulus cell cultures, different effects of PCB congeners were noted (*38*).

For increasing γ-HCH levels, higher follicular estradiol concentrations were noted. As testosterone levels were inversely correlated to γ-HCH, the increase of estradiol levels may be accounted for by a stimulation of the enzyme aromatase converting testosterone into estradiol. In animal feed studies, γ-HCH exhibited estrogenic and antiestrogenic effects. Furthermore, increased as well as decreased blood estrogen levels have previously been noted in association with γ-HCH (*3,39–42*). A direct association was also found between estradiol levels and DDE without any feedback effects on pituitary hormones, which has also been noted in animal studies (*2,43,44*). Direct hormonelike CHC effects on the cellular level and effects on hepatic steroid metabolism may contribute to the confusing variety of CHC-induced hormonal changes (*45,46*).

Prolactin levels were only associated with PCBs. Increasing PCB levels were directly correlated to prolactin levels, which was not due to a TRH-induced prolactin increase, as basal and stimulated TSH levels were rather low. PCB may have a direct stimulating effect on the prolactin-producing cell. The different PCBs may have different cellular effects that account for the varying, partially inconsistent results.

The CHC score was used to evaluate the total CHC body burden. We found significant direct associations to FSH and prolactin and an inverse correlation to testosterone. This may also confirm a specific ovarian and pituitary CHC sensitivity.

We noted numerous immunological changes in association with the different blood CHCs. Increasing PCP levels were associated with decreasing lymphocyte counts and IgG levels. In a previous study of women with exposure to wood preservatives, *in vitro* stimulation of lymphocytes was found to be abnormal and decreased T-suppressor cell counts were detected (*13,47,48*). In cell cultures, abnormal lymphocyte activity was induced with technical- or analytical-grade PCP (*49–51*). We found different immunological changes in association with increasing γ-HCH levels. In women with secondary miscarriages, we observed increasing counts of lymphocytes, especially T-helper cells. We detected increasing counts of T-suppressor/cytotoxic cells, monocytes, and natural killer cells in women with early miscarriages. This may indicate an increase of cytotoxic potential, which may be directed against the fetus. It is known from cell and animal studies that low doses of CHC have a stimulating effect on the immune system, whereas high doses exhibit a suppressive effect (*51–55*). The production of tolerance-inducing factors during early pregnancy may be disturbed due to the activation of the immune system. This may lead to the rejection of the fetus, which immunologically represents allogenic material (*54,56*). In women with secondary miscarriages, increasing counts of lymphocytes and T-helper cells may indicate the induction phase of the immune response, which may lead to an activation of cytotoxic mechanisms and the rejection of the fetus. The increased levels of T-suppressor/cytotoxic T cells and natural killer cells in women with early miscarriages may already represent the cytotoxic activity directed against the fetus.

Increasing HCB levels were associated with decreasing T-suppressor cell counts, resulting in an increased T-helper/T-suppressor cell (CD4/CD8) ratio, especially in women with late miscarriages. Neopterine levels were also decreased. Increasing PCB levels were associated with increased counts of monocytes and natural killer cells and decreased counts of interleukin 2 receptor-positive cells and neopterine levels. The total leukocyte count and IgM levels were also increased. During pregnancy, the count of natural killer cells normally decreases in the eighth gestational week. Activation of natural killer cells may interfere with the immunological tolerance of the fetal allogenic material. Increasing PCB levels in women with late miscarriages were associated with an increasing cytotoxic potential, which was not found for increased HCB levels.

Similar results have been previously observed in animal and human studies. Feed studies with PCB and γ-HCH resulted in dose-dependent changes of immunoglobulins in monkeys (*57–60*). Mallmann et al. (*61*) found a decrease of interleukin 2 receptors in women with repeated miscarriages. As indicated by the varying associations between CHCs and immunological factors,

it is evident that a direct association between one CHC and a specific immunological reaction cannot be found. As humans may have increased levels of different CHCs and other harmful substances, the individual immunological pattern cannot be predicted.

We must emphasize that this is an observational study conducted to detect possible associations between different CHCs and hormonal or immunological changes and to create new working hypotheses for controlled studies. The number of statistically significant findings could well have arisen by chance due to the large number of parameters tested. Because the results partly show similar changes, these findings may indicate real pathogenic and etiological associations, although controlled studies are to be conducted. The results of this study do not indicate that blood CHC levels in women with repeated miscarriages should be routinely determined, but PCP levels should be determined if the individual patient's history reveals exposure to wood preservatives. Animal and experimental studies confirm hormonal and immunological associations with CHCs. CHCs may therefore play an etiological role in the pathogenesis of miscarriages. To reduce additive and potentiating negative effects of environmental harmful substances on the course of pregnancy, women with a history of miscarriages may benefit from consultations in specialized clinics where individual environmental factors can be analyzed and discussed in detail.

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